A SIUDY OF THE DISTRIBUTION OF GLUIEN WITHIN THE WHEAT GRAIN.

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The object of the present paper is to briefly describe several methods for the demonstration of gluten in the central portion of the wheat grain, and the results of their application.

For many years the great majority of observers and of writers upon gluten have stated that this highly important nitrogenous element of food is found almost, if not quite exclusively, in the fourth layer (Parkes) of the grain, immediately below and adherent to the third or inner coat of the true bran; this fourth layer is composed of closely packed yellowish granular cells of ovate or cuboid form, each of which is provided with a dense, laminated cellulose wall and contains a large proportion of free fat. Immediately within this layer of so-called "gluten-cells," and constituting the greater portion of the grain, is an aggregation of much larger, usually elongated, cylindrical cells, whose contents are *apparently* made up exclusively of starch granules which exhibit great diversity in size.

So fixed and widespread has the belief become that the gluten of the wheat resides in specific cortical cells of the grain, that not only do many most intelligent persons habitually rasp their digestive surfaces with branny foods, but attempts to determine, by microscopical examination, the nutritive values of various prepared foods have been made, in which the proportion of "gluten-cells" found in a given food formed the criterion of its value.[1] These assumptions have called forth merited criticism from Prof. Richardson, of this city, and from Prof. Leeds, of Hoboken, both of whom emphasized the fact, singularly ignored by Cutter, Jacobi and their followers, that ordinary white wheat-flour contains a varying but always notable quantity of gluten.

So far as the writer is informed, however, there has not been recorded any ocular demonstration of the gluten of the wheat grain, *in situ* and entirely independent of the "gluten-cells." Such a demonstration may be conclusively made by either of the following methods:

1. If whole wheat grains be macerated in water to which a few

[1] E. Cutter, M. D., Galliard's Med. Jour., Jan., 1882.

drops of ether have been added to prevent germination, they will, in a few days, become thoroughly softened, and the contents of such a grain may then be squeezed out as a white tenacious mass. Examination of the remaining bran shows the "gluten-cells" undisturbed, closely adhering to the cortical protective layers. By now carefully washing the white extruded mass, the major part of its starch may be removed; and upon the addition of a drop of iodine solution, microscopic examination shows numerous networks of fine yellow fibrils, still holding entangled in their meshes many starch granules colored blue by the iodine. In carefully washed specimens, these sponge-like networks are seen to retain the outline of the central starch-filled cells, and evidently constitute the protoplasmic matrix in which the starch granules lay. Upon gently teasing such a specimen under a moderate amplification the fibrils will be seen to become longer and thinner in a manner possible only to viscid and tenacious substances—a class represented in wheat by gluten alone.

An eminently satisfactory proof of the proteid nature of these central networks may be obtained by heating the specimen in the solution of acid nitrate of mercury (Millon's reagent), when the fibrils will assume the bright pink tint characteristic of albumenoids under this treatment. The results of the application of the xanthoproteic and biuret reactions are equally conclusive, but more care is required in the use of these proteid tests, and the resultant differentiation is not so clear. Reticuli similar to those above described, but much broken and smaller, may be seen, upon close examination, scattered throughout fine white flour, without the addition of any reagent.

By general consent, the albumenoids of the wheat grain are grouped together as gluten, which is, however, further separable into gluten-fibrin, gliadin and mucedin, proteid bodies practically equal in nutritive value, but differing in certain physical properties, notably that of solubility. It must, therefore, be borne in mind that in this, as in all other methods of separating gluten from the other constituents of the grain, its relatively small soluble portion is removed with the starch, and that any estimate of the quantity of gluten based upon such methods will probably be rather under than over the actual amount.

2. In even the thinnest sections of the wheat grain, the gluten of the central portion is always masked by large numbers of starch

granules. These may, to a large extent, be removed by immersing the section for a short time in liquor potassæ, with subsequent careful washing. The alkali affects the hydration and partial solution of the starch; but if its application be too long continued, the gluten will also be dissolved. This treatment is well adapted to show the rather dense gluten networks usually found in bran, immediately below the fourth layer.

3. The most satisfactory method of studying the distribution of gluten in sections of wheat is that of *artificial salivary digestion.* If the section be gently boiled for a moment to hydrate the starch, then transferred when cool to filtered saliva, and maintained for from half an hour to an hour at a temperature of about 98° Fahr., all the starch will be digested away, while the insoluble proteid and other constituents will remain entirely unaltered. A section of wheat grain thus treated will exhibit, throughout its entire central portion, close-meshed gluten networks, which become slightly denser toward the cortex of the grain. The proteid character of these reticuli is here, as in the first method, susceptible of micro-chemical demonstration by Millon's reagent or the biuret reaction. A relatively very faint coloration, indicating the presence of albumenoids, is noticeable in the "gluten-cells," while the gradual condensation of the gluten of the endosperm as the cortex is approached, is evidenced by a quite vivid coloration of the fibrils.

Schenk[1] has applied Millon's reagent to sections of wheat with a resultant assumption by the endosperm of a pink tint and "no coloration of the cortical gluten-cells." The starch was not removed and the method of distribution of gluten was not determined. By artificial gastric digestion of wheat sections, the same observer noted that the starch of the section became readily detached, and deduced from this the just proposition that the gluten lay between the starch granules.

Objections are not infrequently offered by the chemist to the microscopical determination of organic compounds, especially where any attempt at a quantitative estimation is made. All that is claimed for the methods above described is the demonstration of gluten in very considerable quantity in the inner layers of the wheat grain. It is but just to state, however, that by these methods a conception may be obtained of the quantity of proteids

[1] Anat.-Physiol.-Unters., p. 32. Wien., 1872.

within the grain fully as accurate as that given by the usual chemical method of estimating the albumenoids of a given body, namely, from the entire amount of nitrogen contained in it. Especially is this true in the case of vegetable tissues. In a close analysis of the potato, Schultze and Barbieri found that only 56·2 per cent. of all its nitrogen existed in albumenoid combination, while in the fodder-beet only 20 per cent. of the nitrogen went to the formation of albumenous compounds; the remainder in each case entering into the composition of non-nutritious bodies, as amides, nitrates, ammonia and asparagin.

The fact that the gluten networks become denser toward the periphery of the endosperm, together with the presence of non-albumenoid nitrogenous compounds in the perisperm, explains the notable percentage of nitrogen found in bran as ordinarily roughly removed.

The color tests mentioned above indicate that the amount of proteids contained in the cells of the fourth layer is relatively very slight; but admitting for the moment that these cells contain gluten, the question naturally arises whether, in view of their dense cellulose walls, they are capable of serving as a food-stuff for man. In artificial digestions the writer has found these elements, even when thoroughly cooked, to be unaffected by the digestive juices; that is, well-boiled bran with its adherent "gluten-cells," will sustain prolonged maceration at the temperature of the human digestive tract in artificial gastric and pancreatic juice (in which, under the same conditions, fibrin is readily digested) without exhibiting any change. These cells were further found to be unaffected by maceration for thirty days in liquor potassæ, except for a slight swelling of the cell and the occasional coalescence of some of its contained oil-globules. They were also practically unchanged by a few days' immersion in strong nitric acid. In order to obtain conclusive and unassailable results as to the nutritive value of the "gluten-cells" as far as man is concerned, the writer has at present under observation a number of healthy adults, who daily receive, in addition to their regular diet, a small fixed amount of boiled bran. Their alvine dejections (containing all the undigested elements of food after the normal action of all the digestive juices) will be submitted to close microscopical examination, with a view to ascertaining the extent to which the "gluten-cells" have been digested, and a report will be made upon the results in the near future.